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ESTIMATION OF SERUM TOTAL PROTEIN LEVELS IN PATIENTS WITH ORAL CANCER- A STUDY

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ABSTRACT

Background and Objectives

The present study was conducted to estimate the serum levels of Protein in patients with oral cancer, compare these values among patients with oral cancer and normal subjects and to correlate the values among clinical and histological grades in oral cancer.

Materials and Methods

The study consisted of 30 oral cancer patients and 30 normal subjects. Diagnosis of oral cancer was based on clinical and histopathologic findings. The patients were grouped clinically according to TNM staging given by American Joint Committee on Cancer and histopathologically as per the Broder's classification. Serum levels of protein were estimated using semiautoanalyser and data was statistically analysed. Data analysis was carried out using Statistical Package for Social Science (SPSS, V 10.5) package.

Results

Study showed no change in serum levels of protein in patients with oral cancer compared to that of normal subjects. The mean serum levels of protein showed no change through clinical stages and histological stages of oral cancer.

Conclusions and Clinical Significance

However our study showed no change in serum levels of protein in patients with oral cancer compared to that of normal subjects. But to validate the above results further studies on large sample size are required.

KEYWORDS: Oral Cancer, Trace Elements, Serum Protein

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Original Article

INTRODUCTION

Despite half century of intensive efforts throughout the world, cancer still remains an enigma. The incidence of head and neck cancer accounts for 30-40% of all malignant tumors in India.[1]Head and neck squamous cell carcinoma ranks 6th world-wide for cancer-related mortality, with an estimated 500,000 new cases diagnosed yearly.[2]

Protein deficiency is common in patients with head and neck cancer and is usually the result of inadequate caloric intake due to local tumor effects, combined with the chronic effects of tobacco and alcohol. [3]

Despite tremendous advances in the diagnosis and management of oral cancers, the diagnostic adjuncts which are used to aid an early diagnosis of oral cancers either suffer from a lack of sensitivity in the initial stages of the processes leading to frank oral cancers or from a setback of not being so cost effective. In addition, biopsy, which is considered the gold standard in the diagnosis of oral cancers, suffers from the reliability of an appropriate site for the obtainment of specimens to be conclusive. The introduction of the concept of field of cancerization has further questioned the significance of biopsy results in the approval or rejection of the reports that come out to be confirmative of either dysplastic or frank cancerous changes seen in the tissue.[4]

The role of biochemical markers, on the other hand, comes out to be a convincing enough evidence of the changes taking place in the body at a time when tissue and cell level changes are not obvious to be taken as an evidence of frank malignant degenerations. Hence, the present study was planned to assess the levels of serum total protein levels in patients with oral cancer which may help in earlier diagnosis and /or prognosis of the lesions.

MATERIALS AND METHODOLOGY

This study included 30 patients with Oral cancer and 30 age and sex matched healthy controls and was conducted in the Department of Oral Medicine and Radiology, Government Dental College, Bangalore. The following inclusion and exclusion criteria were used to select the patients for the study.

- Patients who were both clinically and histopathologically diagnosed with Oral cancer were included in the study.
- Patients who were treated for Oral cancer or cancer elsewhere in the body already previously were excluded from the study.
- Patients who had history of diabetes, hypertension, anaemia, liver or kidney disorders or other systemic diseases and a history of carcinoma elsewhere in the body were excluded from the study.

A detailed case history of the patient was taken and patients were subjected to a thorough clinical examination and same was recorded on a standard proforma. A formal ethical clearance to carry out this study was obtained by the Ethical Committee of the college. A formal informed written consent was also obtained from all the subjects of study group.

30 patients of Oral cancer who were diagnosed based on the history and clinical features with confirmation of diagnosis through histopathological examination were included in the study. Then the patients were grouped clinically in accordance with the TNM staging given by American Joint Committee on Cancer[5]:

T (Size of Primary Tumor)

T1s: Carcinoma in situ

T1: Tumor < 2cm

T2: Tumor < 2cm and < 4 cm

T3: Tumor > 4cm

T4: Tumor > 4cm with invasion of adjacent structures (i.e. through cortical bone, Deep into extrinsic muscles of tongue, maxillary sinus and skin).

N (Cervical Lymph Node Metastases)

N0: No node involvement detected

N1: Single ipsilateral node < 3cm

N2a: Single ipsilateral node < 6cm

N2b: Multiple ipsilateral nodes > 3cm and < 6cm

N2c: Bilateral or contralateral lymphnodes< 6cm

N3a: Ipsilateral node > 6cm

N3b: Bilateral nodes > 6cm

M (Distant Metastasis)

M0: No known metastasis

M1: Metastasis present

Staging

Stage 1: T1N0M0

Stage 2: T2N0M0

Stage 3: T3N0M0; T1,T2 or T3N1M0

Stage 4: T4 any N M0; any T N2 or N3 M0; any T or N, with M1.

The Patients with Oral Cancer Were Grouped Histopathologically According to Broder's Grading[6] as Follows:

Grade I: Well - differentiated (less than 25% anaplastic cells).

Grade II: Moderately - differentiated (25% - 50% anaplastic cells).

Grade III: Moderately - differentiated (50 - 75% anaplastic cells).

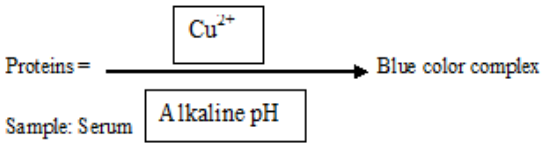
Grade IV: Poorly – differentiated or anaplastic (more than 75% anaplastic cells).

From each subject 5ml of venous blood was collected using aseptic measures from median cubital vein and sent to laboratories in sterile vials for estimation of serum Iron and Total protein levels. The blood was allowed to clot at room temperature for 1-2 hr, then serum was separated by centrifuging at 3000 rpm for 10 minutes and then Serum Total protein levels were estimated using semi autoanlyser.

Serum Total Protein Estimation

Methodology – Biuret method

PRINCIPLE – Proteins react with cupric ions under alkaline pH to produce a colour complex absorbs light at 546 nm (530 – 570 nm). The intensity of the colour is directly proportional to the protein concentration.



Reagent Composition:

Reagent 1 –Total Protein reagent

Cupric II Sulphate: 7mMol/L

Potassium Iodide: 6mMol/l

Tartarate: 20mMol/l

Surfactant: 0.05% W/V

Procedure: Reaction type - End-Point

Reaction time - 5mins. At 37degree Celsius

Wavelength - 546 nm (530-570 nm)

Zero setting with - reagent Blank

Sample volume - 0.01mL (10µl)

Reagent volume - 1.0 mL

Standard concentration - 6gm%

Linearity - 18 gm%

Manual Assay Procedure

Prewarm at room temperature the required amount of working solution before use.

Perform the assay as given below:

Table 1

	Serum/Plasma	Standard	Blank
Working solution	0.01 mL	0.01 mL	_
	1.0mL	1.0mL	1.0mL

Incubation

Incubate the assay mixture for 5 mins at 37 º c. After completion of incubation period measure the absorbance of specimen and standard against blank. Final colour is stable for 8 hours if not exposed to direct light.

Calculation

$$\text{Total protein in gm\%} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times 6$$

Expected value: 6.3 to 8.4 gm %

Data analysis was carried out using Statistical Package for Social Science (SPSS, V 10.5) package. One way Analyses of Variance were used to test the difference between groups and to find out which of the two groups means is significantly different post hoc test of Tukey test was used. In the above test the "p" value of less than 0.05 was accepted as indicating statistical significance.

RESULTS

In patients with Oral cancer the mean age was found to be 54.60±14.85 years (mean ± SD) with 32% in the age group of 50-59 years, 24.3% in the age group of 40-49 years, 18% in the age group of 60-69 years, 19 % in the age group of 70-79 years, 4.3% of patients in the age group of 20 -29 years and 2.3% of patients in the age group of 30 -39 years.

The clinical staging done for Oral cancer as per the TNM staging given by American joint committee on cancer showed that 2 (6.7 %) belonged to stage I, 4 (13.3 %) belonged to stage II, 10 (33.3 %) belonged to stage III and 14 (46.6 %) belonged to stage IV.

The histological grading of Oral cancer patients as per Broder's classification showed that 11 (36.6%) were Grade I, 10 (33.3%) were of Grade II and III, 9 (30%) were of Grade IV.

The mean serum total protein level among the control group was 7.37 ± 0.51 gm% (mean ± SD), and in patients with Oral cancer was 7.15 ± 0.68gm% (mean ± SD). There was no significant difference in the mean serum total protein levels among the study groups. Table 1 There was no stastically significant difference in the mean serum total protein level in the clinically and histologically divided groups in patients with Oral cancer patients. Table 2 &3

DISSCUSIONS

The present study showed no difference in levels of serum total proteins in patients with Oral cancer compared to that of the normal subjects.

Similarly Serum total protein in patients with oral cancer in a study done by Abhishek N et al in 2012[4] came out to be statistically insignificant implying the role of the several complex factors that may play a role in protein metabolism in cancer patients as held by the numerous other studies conducted earlier in this regard.

The analysis of changes in serum total protein in malignancy is in itself a means of studying abnormality in the protein metabolism in this condition. Until recently, radical induced damage to proteins was considered to be mainly a chain-terminating process. It was thought that the products of damage produced on the protein, as a result of protein scission, crosslinking, chemical modification of side chains, were relatively inert with the intermediaries subsequently degraded by intra-and extra-cellular enzymes. It has recently been demonstrated, however, that these intermediaries are capable of initiating further chemical reactions thereby leading to the depletion of important cellular reductants such as ascorbates and glutathione via redox reactions.[4]

Whereas in another study done by Nidhi D et al in 2013[7] showed that mean value of serum total protein too was significantly higher in OSCC patients than in controls. This difference in observations from that of our study could be attributed to different study population and difference in methodology. In the same study salivary protein levels were also assessed which did not show any significant increase in OSCC as compared to the controls. The cause and significance of

this difference is not clear, these salivary proteins are non-specific and their exact significance and role in malignancy is not clearly understood.

Studies have also shown that serum protein biomarkers (gelsolin, fibronectin, angiotensinogen, and haptoglobin,) might be valuable to help differentiate patients with OSCC with lymph node metastasis from those with node-negative disease.[8]

Few studies have estimated serum protein levels in Osf patients. A study of 104 cases of submucous fibrosis by Wahi PN et al showed that total serum proteins, albumins and globulin in patient with OSF were within normal range.[9] But, Pathak AG 1978[10] in his study noticed elevation of total globulins and total immunoglobulins mainly IgG immunoglobulin compared to normal subjects. He stated that these immunologic observations fulfill the criteria of autoimmunity. Another study done by C.D.Anuradha et al in 1993 also showed that there was a significantly increased level of serum proteins in patients with OSF when compared to that of normal subjects. There was also increase in the globulin fraction and a decrease in the A/G ratio. It was stated that this increase in serum proteins might be due to increase in levels of globulins, indicating a state of infection or inflammation. Protein deficiency is common in patients with head and neck cancer and is usually the result of inadequate caloric intake due to local tumor effects, combined with the chronic effects of tobacco and alcohol.[3]The observation of normal levels serum total proteins in our study groups may be related to difference in the method and the study population in our study.

CONCLUSIONS

Detection of cancer at an early stage is of utmost importance to decrease the morbidity and mortality of the disease. Apart from the conventional biopsy, less invasive methods like analysis of serum may provide cost-effective approach for screening a large population However our study showed no change in serum levels of protein in patients with oral cancer compared to that of normal subjects. But to validate the above results further studies on large sample size are required.

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APPENDICES

Table 2: Comparison of Mean Serum Total Protein Levels among Study Groups

	N	Mean	Std deviation	T	P
Control	30	7.370	.519		
Oral cancer	30	7.153	.689	1.37	0.17

Table 3: Comparison of Mean Serum Total Protein Levels among the Clinical Stages in Oral Cancer Group

	N	Mean	Std Deviation	Minimum	Maximum	F	P Value
StageI	2	7.350	.212	7.2	7.5		
Stage II	3	7.100	.173	6.9	7.2		
Stage III	12	7.108	.582	5.8	8.1	.075	.973
Stage IV	13	7.177	.9048	6.0	8.7		

Table 4: Comparison of Serum Total Protein Levels among the Histological Grade in Oral Cancer Group

	N	Mean	Std Deviation	Minimum	Maximum	F	P Value
Grade I	13	7.262	.588	6.0	8.1		
Grade II & III	10	7.130	.713	5.8	8.7	.356	.703
Grade IV	7	6.986	.884	6.0	8.3		